EXHIBIT 2

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What can we learn about human immunodeficiency virus infection from a study of lymphocytic choriomeningitis virus?

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Summary: The role of cytotoxic T lymphocytes (CTL) in human immunodeficiency virus (HIV) infection remains elusive. Since the discovery 10 years ago of high levels of specific CTL in this disease, some have argued that they play an important role in virus control, others that they drive disease progression through destruction of T helper cells, and others still that they play no obvious role at all. By contrast, the central role of CTL in murine lymphocytic choriomeningitis virus (LCMV) infection has been very clearly worked out through the use of *in vivo* depletion and adoptive transfer experiments, as well as knockout and transgenic mice. To interpret the possible roles for CTL in HIV, we have therefore made a comparison between what is known about CTL and their interaction with virus-infected cells in these two infections. This illustrates a potential critical role for these cells in both control of HIV replication and immune-mediated pathology, but one that is highly dependent on virus dose, distribution and dynamics.

Introduction

Why compare LCMV and HIV?

Despite enormous effort, the basic pathogenesis of human immunodeficiency virus type 1 (HIV-1) is still poorly understood. We now know a great deal about the basic molecular biology of the virus, its mechanisms of cellular entry, its dynamics and its evolution within individuals and populations. What is still missing is a description of how persistent infection is maintained in the presence of active immunity, and what the relative roles of the immune system and the virus itself are in the eventual outcome.

The situation in lymphocytic choriomeningitis virus (LCMV) infection in the mouse is very different. For a start, this infectious model has been established for over 60 years (1, 2). The *in vivo* roles of specific immune subsets in the clearance of virus and the induction of disease are well understood. The mechanisms which allow particular virus strains to establish persistent infections have been dissected in fine detail, in particular with the use of transgenic and knockout mice.

On the surface, there is little in common between LCMV and HIV as infectious agents. The former is an arenavirus which

Table 1. Basic characteristics of LCMV and HIV

Characteristic	LCMV	HIV
Virological		
Family	Arenaviruses	Lentiviruses
Host	Mice, hamsters	Man
Target organ	Lymphoid, CNS	Lymphoid, CNS
Target cell *in vivo*	Macrophage +/- lymphocyte	Macrophage + CD4 T cells
Cytopathicity *in vivo*	No	?
in vitro	Yes	Yes
Eclipse phase	10–12 h	8–12 h
Persistence	Depends[a]	Usually
Variability *in vivo*	Mutable	Highly mutable
Immunological		
Induction of CTL	Early	Early
Levels of CTL	High	High
Persistence of active CTL	High	High
Exhaustion of CTL	Yes	?
CTL escape mutants	Depends[a]	Yes
Immunopathology	Yes	?
Neutralising antibody	Late	Late
Binding antibody	Early	Early
Privileged sites	Yes	?

[a]on mouse, viral strain and/or dose

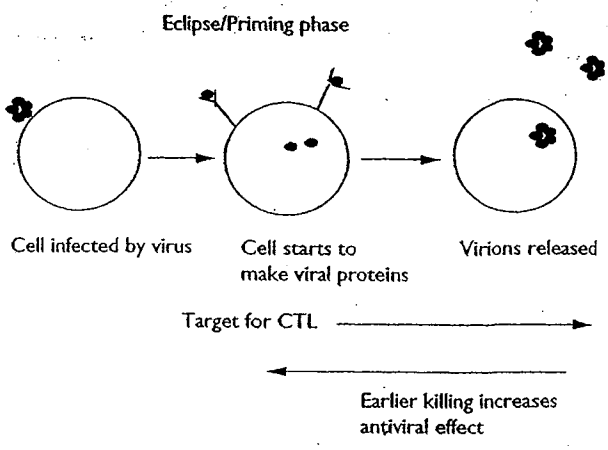

Fig. 1. Distinction between CTL-mediated target lysis and antiviral effect. CTL may lyse targets before they release new virions. For this reason, the effect on virus release is not simply proportional to the rate o destruction, but depends on the amount of "head start". Paradoxically, larger amounts of cell killing may be accompanied by poorer control of replication, the overall picture being determined by the cytopathogenicity of the virus.

normally has as its host the mouse and hamster (although it can cross species into man and monkey). The other viruses in this family include a variety of pathogens causing acute haemorrhagic fevers in man, notably Lassa fever (3, 4). HIV is a retrovirus of the lentivirus group, which causes few immediate clinical symptoms but sets up a persistent infection which leads over many years to immunosuppression and death through opportunistic infection or malignancy.

The reason for embarking on such a comparison is that the dominant immune response to both viruses is the cytotoxic T lymphocyte (CTL), and particular features of this immune response have striking parallels in the two infections. Since the CTL response to LCMV has been studied in immense detail, and its role in vivo has been accurately determined both qualitatively and quantitatively, it provides an excellent reference point from which to view the role of the same cellular response in HIV. A number of important phenomena have been first identified in LCMV and a comparison with HIV is made in Table 1. The discussion below is based on these phenomena – they provide a framework for understanding features of HIV which are currently poorly defined and also for future investigation of this and other persistent viral diseases in man.

What can CTL-mediated killing actually achieve?

CTL, through killing, are only capable of destroying cell already infected with virus. Once an infection is establishe therefore, the ability of a virus to persist in the face of CT depends on a simple balance in which the rate of virus produc tion is at least equal to the rate of clearance. Viruses infectin cells are initially immunologically silent during a so-calle "eclipse" phase prior to new viral protein synthesis (Fig. 1) Subsequently, there is a phase of new protein synthesis befor progeny are formed; during this time, viral antigens passe through the cellular antigen-processing and presentation path way will sensitise these cells to recognition and lysis by CTL - this period may be termed the "priming" phase. Subsequentl new virus particles will be formed, at which point the cell wil remain a target for CTL until its death and the virions will serv as targets for neutralising antibodies.

The effects of CTL-mediated killing in LCMV and HIV ar therefore, importantly, 2-fold: they kill infected cells and the reduce virus production. The amount of reduction in viru depends on the length of the priming phase, the rate of viru production and the rate of killing by CTL (5). Because the vira clearance process is essentially a race (i.e. CTL killing vs. viru

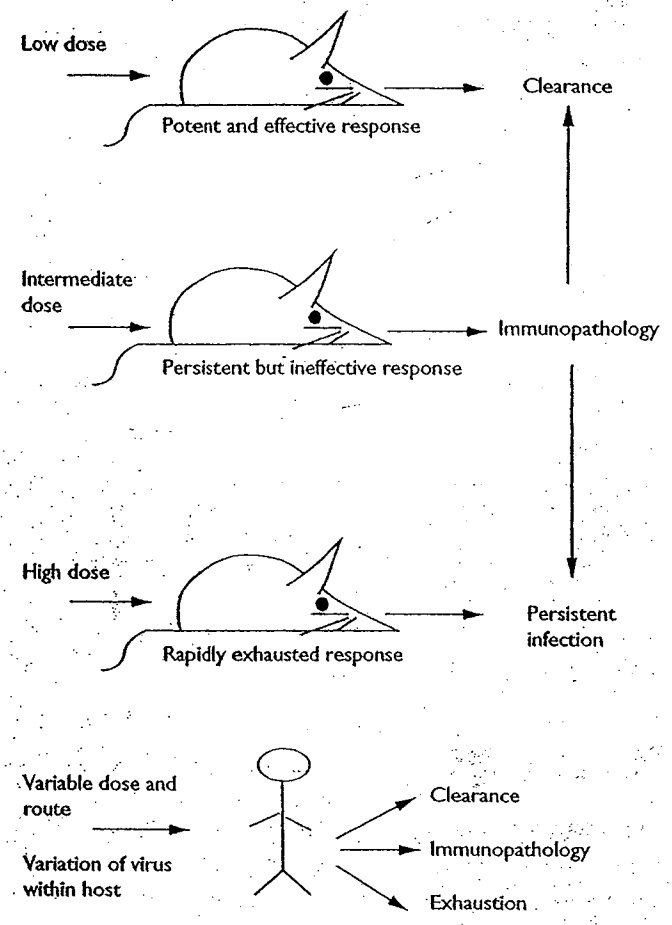

Fig. 2. Possible outcomes in LCMV and HIV infection. Small changes in dose lead to markedly different outcomes of infection, despite induction of a similar initial immune response. Alternatively, the initial immune status of the host may determine the effective dose. Parallel outcomes may be considered in HIV.

production), whereas tissue destruction or immunopathology is cumulative, relative failure in killing may lead to a reduction in virus clearance, but, paradoxically, little reduction in immune-mediated damage. This concept is as valid for HIV as it is for LCMV, and forms an underlying theme through this review (Fig. 2).

Basic virology of LCMV and HIV

Life cycle – both viruses primarily infect lymphoid tissue

LCMV is a bisegmented single-stranded RNA virus with an ambisense genome (6). The L strand contains the polymerase gene and a gene bearing a zinc finger motif, whose function is currently unknown; the S strand encodes the glycoproteins GP1 and GP2, cleaved from a precursor, GPc, and the nucleoprotein (NP) (6). Thus, it is a relatively simple genome, and, in immunological terms, its recognition is dominated by the S strand, which encodes important CTL epitopes in GP1 and NP, and sites for neutralisation by antibody in GP1.

Under laboratory conditions, LCMV is infectious via the intravenous, intraperitoneal or subcutaneous routes, although natural infection is probably spread via saliva, urine or ingestion, and it may cross species into man and monkey (3, 7). The virus replicates in a variety of cell types, with preferences depending on viral strain, which in turn have been correlated with single amino acid changes in GP1 (8–10). However, one consistent site of primary replication is the macrophage, a feature it shares with HIV. Other cell types in which replication has been demonstrated include both CD8 and CD4 T cells, dendritic cells, neurones and glial cells (10, 11). The only absolute requirement appears to be a cell nucleus, without which the virus does not bud efficiently (12).

The life cycle of HIV has been explored in immense detail and has been reviewed widely. By comparison with LCMV, it has a much more restricted host cell range, determined primarily by the presence of a binding site on CD4 (13), and, it is now clear, by the ability thereafter to bind chemokine receptors (14, 15). Initial infection is commonly with macrophage-tropic strains, even from individuals where these are in the minority of the quasispecies, and, during the clinical latent phase, is concentrated in lymphoid tissue (14–17). Nevertheless, its distribution, particularly in late-stage disease, may be quite widespread, including apparently CD4-negative cells such as CTL (18).

Cytopathic effect *in vitro* but case not proven *in vivo*

A key feature of LCMV is its ability to infect cells without significant cytopathic effect. *In vitro*, upon initial culture, there are some cytopathic effects which may be observed (19). However, *in vivo*, this does not appear to be the case as is clear in carrier mice with a deficient immune response: rare functional defects have been reported, although in general any pathology seen in this infection *in vivo* is immune-mediated.

The question as to what extent HIV isolates are cytopathic *in vivo* has been debated and clearly depends on both isolate and host. That certain strains of virus are strongly cytopathic *in vitro* in certain cell lines (for example, T-cell-tropic viruses in C8166 cells) is without doubt, although, even in these cases, changes in culture conditions may dramatically affect cytopathogenicity (20). The relatively constant and short (36 h) half-life of CD4 cells *in vivo*, regardless of the stage of the disease and therefore antiviral immunity, has been taken to indicate that lifespan is

determined by virus and not by host (21–23). The latter calculations may, however, be weighted to reflect the larger amounts of virus produced by cells killed by virus as opposed to host immunity (for example, CTL) (5, 21). There probably exists a biological spectrum of cytopathogenicity *in vivo* – the switch from macrophage-tropic non-syncytium-inducing (NSI) to T-cell-tropic syncytium-inducing (SI) strains is well documented and now understood on the basis of second receptor specificity.

Interestingly, simian immunodeficiency virus (SIV) isolates are not equally pathogenic in all primate strains. This forms an interesting comparison with LCMV in that the latter is non-cytopathic in its natural hosts – Old World rodents such as mice and hamsters, but certain strains may be highly virulent in New World rodents such as guinea pigs. (3).

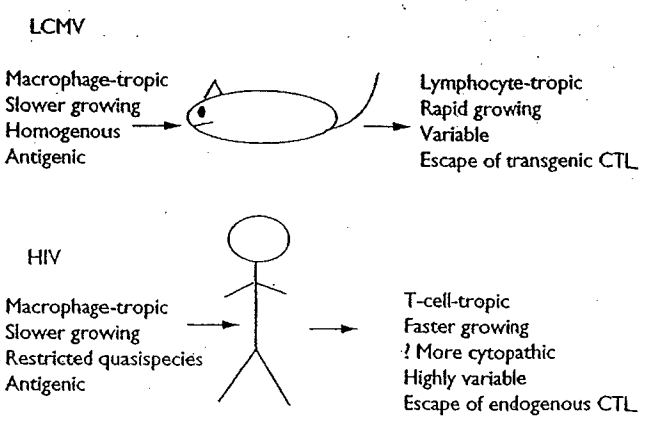

Fig. 3. Evolution of viruses within the host. LCMV is normally cleared, but may persist and evolve under various conditions (see text). HIV is normally not cleared and may change genotype and growth characteristics and mutate epitopes.

An extended priming period leaves virus-infected cells open to destruction

Studies of the life cycle of LCMV *in vitro* indicate an intracellular phase requiring 8–10 h for new virion formation, and, *in vivo*, a similar eclipse period is seen (S. Ehl et al. & P. Klenerman et al. submitted). This compares with lytic viruses such as vesicular stomatitis virus (VSV) or vaccinia, where much earlier virus bursts are observed (24).

The intracellular life cycle of HIV is more complex than that of LCMV and is divided into an initial phase of reverse transcription followed by nuclear transport, integration, transcriptional upregulation and new virion formation, leaving an extended priming period (25). One relevant feature in HIV is the early formation of regulatory factors such as tat, rev and nef prior to the synthesis of structural proteins and new virion formation. Thus, the recognition of regulatory proteins in CTL targets might prolong the priming phase, although the relative protective capacities of CTL of different specificities *in vivo* have not been addressed.

Both viruses evolve during infection *in vivo*

The ability of LCMV to mutate under selection pressure has been demonstrated *in vivo* (26) and *in vitro* (27). Certain strains isolated from carrier mice show enhanced tropism for lymphoid tissue and more rapid growth (8–10). Mutation will depend on the fidelity of the polymerase gene and the rate of viral replication (28), although, under normal conditions of infection, the virus remains relatively homogeneous (Fig. 3).

Transcriptional errors are common in HIV and are generated by a variety of mechanisms (29). The consequences of such mutation depend on the local replicative advantages of the mutant virus, a proportion of which will depend in turn on the local immune response. The original scheme shown in Fig. 1 is an aid to understanding the selection pressures placed on such viruses by CTL lysis, which are very different to those placed by drugs. Since CTL act to reduce the rate of production of new viruses by cells that are already infected, rather than by blocking one particular step of the life cycle, mutant viruses will have only a relative advantage. This is illustrated *in vitro* for LCMV, where mutant viruses with reduced recognition by CTL are only efficiently selected in the presence of extremely high rates of killing by CTL (27). CTL which act through virostatic cytokine secretion or chemokines (30) which block viral entry to cells (31, 32) could be envisaged as having a selection effect more akin to drugs.

CTL response against LCMV and HIV

The acute CTL response is extremely vigorous

The acute response and initial control phase in both LCMV and HIV is dominated by CD8-positive MHC class I-restricted CTL. The role of these CTL has been amply demonstrated in the mouse (24, 33–38). This is efficiently performed through lysis of infected cells, since perforin gene knockout mice are unable to control virus replication, despite generation of antiviral CTL (39–41). These mice ultimately die of cytokine-mediated immunopathology – a variation on the theme of CTL-induced death.

In C57BL/6 mice, after intravenous infection with the WE strain of LCMV, the CTL response is difficult to detect *ex vivo* during the first few days of infection, but peaks at day 8; this coincides with the maximal rate of viral clearance under normal cir-

cumstances. The numbers of effector cells can be measured using a limiting dilution assay, and, at this stage, have been measured as approximately 1/100 (42). This value, although extremely high, however probably underestimates the true number of effector cells, due to death *in vitro* during the period of restimulation. Alternative methods of quantitation of these effectors include dilution of the *ex vivo* effector response and comparison with lysis by cloned CTL. A yield of 60% specific release in 4 h at an E:T ratio of 1:1 by the latter is equivelant to that of *ex vivo* splenocytes at an E:T ratio of 10:1 (43, 44). This would imply that roughly 1 in 10 splenocytes or up to 1 in 3 CD8 cells could be specific for a single epitope at the peak of the anti-LCMV response.

The levels of acute effectors decline rapidly as the virus is brought under control, and only a week later become virtually undetectable by standard *ex vivo* assays: the precursor levels remain, however, high (45). This has two important implications for human studies: firstly, that responses may fluctuate extremely rapidly (and therefore that negative responses must be interpreted with care); and, secondly, that there is not a direct correlation between precursor frequencies and *in vivo* effector function, even at time points a week apart.

How is this reflected in HIV? Some analysis has been made of the levels of CTL and their activity during the acute stages of infection – notably this has been found to occur before neutralising antibody is detectable. Fresh *ex vivo* lytic activity has also been observed (P. Klenerman & D. Price, unpublished observations): previous estimates have suggested that such killing is only seen in the presence of CTL effectors at a level of about 1 in 10 PBMC (44), i.e. similar to that observed in LCMV.

The important point about such observations is that of the temporal association between the appearance of CTL and the initial control of viraemia (46, 47), although it is suggested that in fact the viraemia may terminate independently from the immune response due to reduction in available targets (48). Significant expansions of CD8-positive T cells with particular V beta usages have also been observed during the acute phase of disease. Some of these were associated with specific antiviral CTL activity after *in vitro* culture, although this was relatively weak, considering the degree of expansion *in vivo* (49). The latter may reflect once again the difficulty of growing such committed effector cells *in vitro*, the relatively transient nature of such massive early responses as seen in LCMV, or the development of immune escape variants (see below).

Such very high levels of CTL are by no means restricted in man to HIV, since similar responses occur in Epstein-Barr virus (EBV) during the onset of acute symptoms (50). Acute influenza infection also induces expansions of CTL with restricted TCR usage depending on HLA type of the individual, and, briefly, the capacity to kill directly *ex vivo* (51).

A further feature of note is the influence that this initial response has upon the subsequent course of the disease: it has been observed that viral load early after seroconversion determines subsequent rates of disease progression, a process which may take several years. Furthermore, the breadth of T-cell activation, as determined by the number of V beta families showing expansion, also determines subsequent CD4 decline (the broader, the slower) (49, 52). Since it appears from LCMV that small changes in the efficacy or dynamics of CTL can have large influences on ability to control infection, it may be that many of the critical events determining outcome are decided within the first few days of infection.

Active CTL persist at high levels after the initial control of viraemia

Antiviral CTL remain at an elevated frequency for many months after initial LCMV infection (42, 45). These cells apparently possess immediate (protective) effector activity since they kill *ex vivo* in assays of extended length (10–15 h) (Fig. 4A). Such activity has been taken to indicate continued exposure to viral antigens; the known capacity of the virus to persist in relatively privileged sites such as the kidney may well contribute to this. Thus, this level of CTL could continuously mediate a low-grade clearance of virus.

Examples of the difficulties in translation of CTLp frequencies into *in vivo* effect are found during this phase. Mice deficient in CD4 cells (53) may show similar CTLp frequencies to controls after initial clearance of low-dose virus, but dramatically different subsequent disease courses (see below). Similar CTLp frequencies are also found in mice 20 days or 200 days after infection, but, upon adoptive transfer, the cells show much more efficient protection earlier after infection (S. Ehl et al. submitted).

In HIV, a similar situation of active circulating CTL (Fig. 4B) and elevated precursor frequencies is seen, although, in this case, it is clear that continuous virus exposure is taking place (54). There is no fixed relationship between either *ex vivo* killing or CTLp and virus load or disease progression (55, 56). Because the methods used for *in vitro* restimulation may require T-cell help or professional APC, it is possible that loss of CTLp activity in later HIV may be exaggerated by the use of LDA (57). One study found that *ex vivo* responses, measured as lytic units, persist in patients as their disease progresses, while CTLp measured as LDA dropped, consistent with this idea (56). Since measurement of *ex vivo* responses is quite insensitive, this situation may actually be more common than initially thought. Sen-

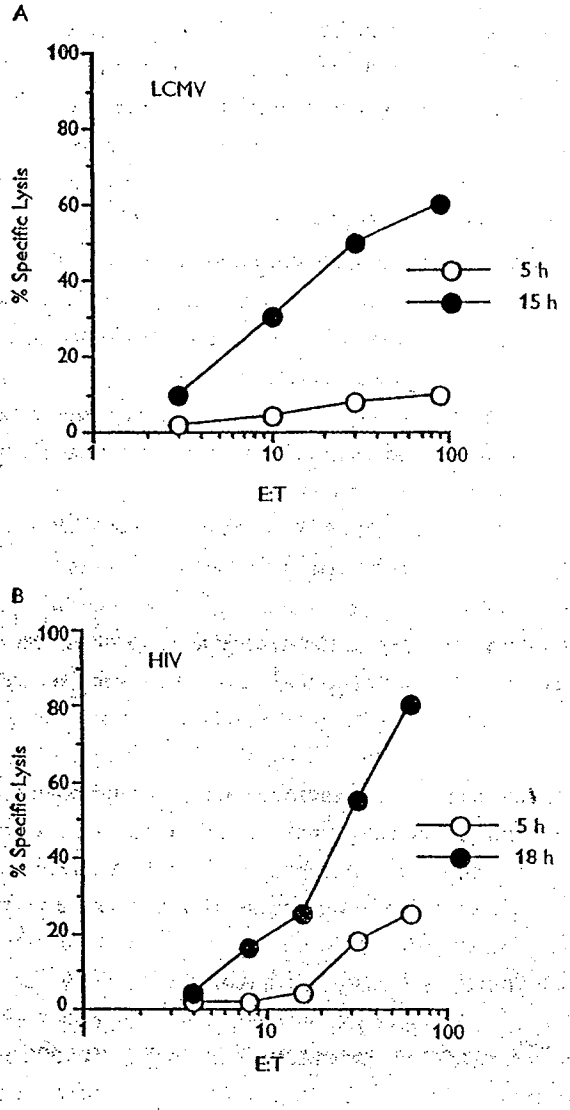

Fig. 4. A. CTL responses in memory phase. Freshly obtained splenocytes from mice 50 days after infection: lysis of targets sensitised with GP33 immunodominant epitope. **B. Fresh *ex vivo* responses in post-acute phase.** CTL assay from PBMC of asymptomatic individual directed against HLA B8-restricted gag epitope (p17-3).

sitive measurement of such populations is now possible using ELISPOT or FACS staining using MHC-peptide complexes, and the results of longitudinal studies using such methods will be of interest (58).

In both diseases therefore, there is continuous activity by effector CTL. The main difference here is the level – in LCMV, virus may be present only at extremely low levels, analogous to patients with extremely well controlled HIV infection (59); for example, those on multiple drug therapy. In other patients, the same or greater levels of CTL may be engaged in continuous cellular destruction in an unsuccessful attempt to clear virus.

Exhaustion of CTL is seen in LCMV and probably occurs in HIV

The loss of killing activity is central to the issue of CTL in control of viruses. The phenomenon of exhaustion was first demonstrated in LCMV, and has been proposed as a mechanism for CTL decline in HIV (42, 60, 61). Exhaustion describes the decline in CTL number and activity after overwhelming stimulation by virus *in vivo*. This may occur in normal mice using specific variants such as LCMV-Docile strain, which replicates widely and rapidly *in vivo*, but is cleared as for more commonly used strains when given at low dose (200 pfu iv). In higher doses (10^{5-6} pfu intravenously, but not subcutaneously), it is not cleared, and causes a persistent infection; a similar phenomenon is seen with LCMV Clone 13, an Armstrong-derived strain cloned from persistently infected mice (11). Under these circumstances, CTL are induced and killing can be observed in the initial phases, but their activity does not reduce the peak viraemia. They subsequently disappear from lymphoid tissue and are not detectable, even after restimulation *in vitro*.

In other strains, exhaustion can be induced by tipping the virus:host balance slightly in favour of the virus. For example, CTL exhaustion is more readily observed in mice deficient in CD4 help and, interestingly, in those deficient in B cells (62). In both of these cases, high doses of WE strain (which did not normally induce exhaustion) produced an effect similar to Docile. Exhaustion may also occur upon adoptive transfer of CTL into persistently infected WE carrier mice, again a situation in which effector cells are confronted with a relatively high load of virus replicating in a range of sites (63). Thus, one feature of exhaustive conditions, if not the complete explanation for the phenomenon, is either a distribution or replication rate of virus which is sufficient to stimulate CTL responses but in excess of their capacity to control infection.

An association has also been made between virus strains which efficiently infect lymphocytes and those which induce a persistent infection. The numbers of lymphocytes infected are quite low (2–3%), and this does not yet provide a complete explanation for a selective loss of activity (11). However, a simple mathematical model predicts that exhaustion is likely to take place under conditions in which the virus strain is rapidly replicating and also has the capacity to infect lymphoid cells – indicating that perhaps both components are required *in vivo* (D. Wodark et al. submitted); exhaustion may also occur due to persistent infection of the periphery, where CTL may be effectively "starved" of supportive cytokines (62). Small changes in

replicative ability as seen in LCMV strains could have a large influence on exhaustive capacity and thus on establishment of a carrier state.

The issue of exhaustion in HIV has been touched upon above in the disappearance of the acute expansions of CD8 T cells. The conditions under which exhaustion has been best studied in the mouse have been during this acute phase, although this is technically demanding in man since patients rarely present at the very earliest stages after infection. It is still possible that significant responses to certain epitopes appear and disappear at this stage – before simple CTL measurements have often been made. This was indeed observed in 2 seroconverters studied at a very early time point, whose original anti-nef and anti-gag responses, respectively, were not obtainable later than 2 weeks after presentation (D. Price et al. in preparation). The huge number of epitopes potentially recognised in HIV and the large interpatient variation would mask this effect. It may be that a number of effective CTL responses are lost during this crucial early phase.

If a prediction is to be made from the LCMV studies, it would be that those infected by the intravenous route may show a greater tendency towards early exhaustion than those infected by more standard mucosal routes, although doses and co-infectious agents also differ between these groups. Vaccination against mucosal infection may also be easier than against blood borne, for the same reason.

The major issue in HIV is the long-term disappearance of CTL responses. Some decline in the number of specific TCR has been observed over time in some studies (64), but not in others (65). Recent studies of CD8 turnover in HIV using analysis of telomere length have suggested that CD8 turnover is extremely high, a feature which could contribute to long-term loss of cells (66). There is also some *in vivo* evidence for CD8 apoptosis in pathogenic but not non-pathogenic strains seen in SIV models (A. McMichael, personal communication).

Thus, CTL exhaustion in HIV may occur in the acute phase in an analogous manner to LCMV, or as a more chronic process in the long term. The requirements for exhaustion as defined in LCMV are extensive replication, rapid turnover of virus, infection of lymphohaemopoietic cells (plus probable extensive replication in peripheral organs) and a vigorous initial CTL response – HIV is well qualified in all these areas.

Escape mutation is readily seen – under appropriate selection

In LCMV-WE infection of B6 mice ($H2^b$), there are strong CTL responses to three main epitopes on the S strand. Although the virus has the capacity to vary and replicates to high levels, under normal circumstances, immune escape is not seen. Viruses which have mutated two out of three of these major epitopes are also cleared, albeit at a slightly reduced rate (67). Thus, viruses which have through chance eliminated recognition of only one of these epitopes through genetic mutation do not possess a strong selective advantage *in vivo*. Mutation of all three major epitopes during the relatively short period of CTL clearance of virus seems to be too rare an event, and there may yet be other epitopes which are recognised lower in the hierarchy of immunodominance (68). Thus, under normal circumstances, immune selection and viral escape do not play an important role in CTL control of LCMV.

This contrasts markedly to situations in which the immune response is more tightly focussed on one individual epitope. This is best illustrated *in vivo* by the TCR-transgenic mouse bearing a receptor specific for the dominant epitope GP33 (26). While the level of the response as measured *ex vivo* is no higher than that seen in control mice, mutant viruses under these circumstances possess a significant selective advantage and come to dominate the population, which then escapes the initial immune response (Fig. 5). Similar mutant viruses may be selected by similar means *in vitro*, given a high enough level of killing (27). Interestingly, in the case of the GP33 mutant, the mutation does not affect binding to MHC (which would be the most effective means of immune escape) since it is recognised by a polyclonal T-cell response – that is, it falls into the group of altered peptide ligands (APL) for CTL (69, 70). The role of viral APL *in vivo* and the results of their ability to partially activate CTL are currently under study in the LCMV system.

One further issue in LCMV is the role of antigen dose in the generation of virus escape. Viruses inoculated at low dose may be cleared efficiently, and, even under conditions of monospecific immunity, fail to generate *in vivo* escape mutants (26, 27). This neatly encapsulates the point that generation of viral escape mutants depends on the rate of replication of the virus, and is probably critical in HIV (71).

The relevance of mutations in epitopes in HIV has been debated since they were first described, and this issue by analogy depends on the restriction of the immune response both between and within epitopes (72, 73). A very clear example has recently been provided by Goulder and colleagues (71), where a long-term response restricted to a single epitope in p24 gag, dominated by a single clone of T cells, was ultimately associated with the production of a mutant epitope, and escape of the virus *in vivo*. Similar escape at the time of seroconversion, again associated with a highly focussed response against an epitope in envelope, has also been observed (74, 75). In general, mutations which arise and are not recognised by the ongoing immune response in *in vitro* assays have not been so

obviously associated with loss of control of the virus *in vivo*; it is possible that shifts in immunodominance compensate for loss of CTL recognition of a particular epitope (68).

Do the immune responses in HIV resemble more closely the situation in the wild-type or the TCR-transgenic mouse? This question has not yet been fully resolved. There is some evidence from the V beta expansions in acute disease that there may be early restriction of TCR usage (49). Long-term responses may show quite remarkable focus, with single clones dominating the entire antiviral response over many months (65). Although the potential breadth of the response is clear, and multiple epitopes restricted by a variety of HLA types are seen in several genes, direct *ex vivo* measurement of individuals' responses over time has rarely been done. HLA type may play an important part, directing dominant responses to relatively conserved or variable epitopes.

APL also dominate the list of variants seen to escape from CTL in HIV (76–78). A proportion of these have been shown to act as TCR antagonists *in vitro* (78), a feature also seen in variants arising in hepatitis B (79). Whether these act as antagonists *in vivo* will depend not only on the restriction of the immune response, as appears to be the case for escape mutants in general, but also on the proportions of the variants. If the rates of lysis are critical, then even relatively small effects may ultimately be important, as is potentially the case in HIV (5).

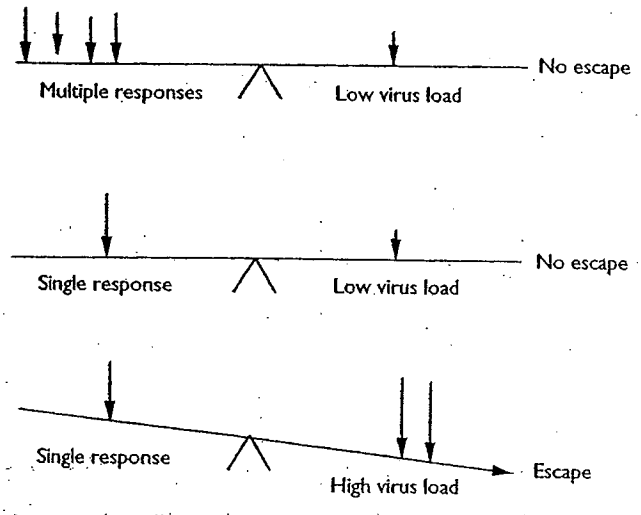

Fig. 5. Immune escape lessons from LCMV. CTL escape mutants only come to force under conditions of a monospecific CTL response and relatively high rates of virus replication. The same may be true for HIV.

Although such effects are potentially quite powerful, they h not been proven to occur *in vivo*.

CTL-mediated clearance of virus depends on distribution of infection

In LCMV, the requirements for CTL activation and cytolysis well understood *in vitro*, and also *in vivo* within a spleen or lym node. By contrast, little is known about the ability of CTL perform their tasks in the diverse environments required fro them in a widespread viral infection. The same rules may sir ply not apply, as is evidenced by the fact that antigen presen tion by the same cell may have very different outcomes depen ing on whether this takes place in the spleen or in the periphe (80). CTL in LCMV, while extremely effective in clearing lyr phoid tissue, and also lung and liver, are functionally deficie in clearing the kidney, for example (67). In the acute phase disease, the kidney is cleared much more slowly than oth organs, including the spleen (which is a major site of virus re lication). Furthermore, during the phase of long-lived imm nity, virus may persist in the kidney, even after low-dose infe tion, in mice where no replicating virus is detectable elsewhe (7). The reasons for this are not clear but could lie at the lev either of antigen presentation or simply of access.

Interestingly, mice which are deficient in B cells or in help are particularly susceptible to persistence of virus. Indee mice may be free from detectable viraemia for several weeks months before it reappears (63, 81). It seems that antiviral ant bodies are important in the long-term control of replicatic possibly through their action in diverse sites, where CTL co trol is less effective. As described above, CTL, through killing infected cells alone, are only really capable of reducing the ra of virus production from cells already infected. In the case an established infection therefore, it seems clear that oth mechanisms may be required in addition. Finally on this poir CTL activity is lost as virus recrudesces in such help- or ant body-deficient mice, although memory as established *in vitro* intact prior to this point. The recrudescence does not appear be due to viral mutation in those isolates tested (P. Klenerma & R. M. Zinkernagel, unpublished observations) and therefo the mechanism for this escape remains unclear, although wi interesting implications for HIV.

The issue of antigen geography has not been fully explore in HIV, although late in the disease there is spread from t lymphoid tissue, which is the major site of replication durir the clinical latent phase, to widespread sites, and also marke variation in distribution within organs (82). Interestingly, or of the variables distinguishing long-term non-progresso from those with progressive disease was the presence of stror

CTL responses – together with neutralising antibodies (83, 84). In the non-progressor patients, the disease is of a very restricted distribution and may be very difficult to detect even by PCR. It is possible that treatments or interventions which induce neutralising antibodies, while having little direct influence on the ongoing disease, may allow for a more sustained antiviral effort by CTL. Lower levels of virus turnover, as seen in LCMV, will also reduce the chance of generating escape mutants, even with a highly focussed CTL response. Finally, the ability of CTL to effectively clear virus replicating in the CNS has not really been addressed, although the biology of such CNS-tropic strains is now better understood.

CTL-mediated damage of lymphoid tissue in both diseases?

In LCMV, the mediators of disease are CTL – whether this is in the meninges, the liver or the lymph node (85–94). Destruction of the APC and lymphoid architecture by CTL-mediated lysis can lead to functional immunodeficiency during acute disease (95, 96). Control of virus replication or exhaustion of CTL limits the duration of this damage and does not lead to chronic immune dysfunction. However, the effects can be relatively long-lived depending on the dose and strain used. Docile, a rapidly replicating lymphotropic strain, induced the most profound lymphoid damage and most effective immunosuppression out of those strains tested (95).

One specific feature of such CTL-induced lysis of lymphoid cells appears to involve the destruction of infected B cells secreting neutralising antibody (87, 97, 98). Hybridomas secreting neutralising antibody, but not non-neutralising antibody, have been found to bear infectious virus *ex vivo* (87). Although this has not been proven *in vivo*, it seems likely that lysis of such cells by CTL might explain the relatively late appearance of such neutralising antibodies in LCMV – a feature it shares with HIV.

Are such mechanisms likely in the latter? Few attempts have been made to address this directly. Certainly CTL are present in high numbers, in the correct location, and faced with infected immune cells (macrophages and CD4 cells) as targets. We have recently shown that the efficiency of killing by CTL from HIV patients is similar *ex vivo* to that seen in LCMV (P. Klenerman et al. submitted). Furthermore, an analysis of HIV turnover *in vivo* coupled with estimates of half-life of infected cells after exposure to CTL indicates that the CTL levels seen in HIV are at least capable of contributing to the very rapid turnover of CD4 cells seen in this disease (5). Persistent virus production and chronic CD4 lysis by CTL over many years may lead ultimately to elimination of both subsets, at a rate dependent on their replenishment. In terms of virus load, it is preferable for the host for cells to be lysed as early as possible by CTL after infection. A certain level of virus may therefore be contained by CTL, with minimal tissue damage, and no increase in virus load; anything which disturbs either the initial setting of this equilibrium or subsequent rate of killing by CTL may lead to a vicious circle where there is poorer control of virus production and increasing levels of tissue damage. Assuming such a situation is true, exactly what the requirements for this balance are in terms of CTL activity, virus load and distribution remain to be determined.

Conclusion – two diseases linked by a common immune effector mechanism

LCMV infection in the mouse has important parallels to HIV in humans, despite the very different structure and replicative strategy of the two viruses. Both viruses induce a substantial CTL response which dominates the early stages of infection and probably determines the ultimate outcome. In this review, we have analysed the role of CTL-mediated lysis in control of virus replication and in destruction of lymphoid tissue in these two viruses and pointed out the features which appear to be critical. These are particularly dose, distribution, and replication rate of the virus at the very earliest stages of disease.

In the mouse, it is possible to prove that such features are relevant *in vivo* by performing the appropriate experiment. In man, we rely usually on careful observation of the natural disease and the results of therapeutic intervention to draw inferences about the true role of these cells. However, it may be possible to address some of the questions about the basic biology of the CTL response using the murine system, and, conversely, many issues which have already arisen out of the murine studies may be highly relevant to the human disease. In particular, immune interventions such as adoptive transfer of CTL have been carefully studied in the mouse, and their conclusions could well be relevant to current human studies.

Overall, it seems that quantitative as much as qualitative aspects of the CTL response are central to the understanding of the issues surrounding antiviral control *in vivo*. Viruses have much to teach us about immunology (99) – but they may also teach us about other viruses.

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